

Molecular characterization of SPM-1, a novel metallo- β -lactamase isolated in Latin America: report from the SENTRY antimicrobial surveillance programme

Mark A. Toleman^{1*}, Alan M. Simm¹, Tanya A. Murphy¹, Ana C. Gales², Doug J. Biedenbach³,
Ronald N. Jones^{3,4} and Timothy R. Walsh¹

¹Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD, UK;

²Division of Infectious Diseases, Universidade Federal de Sao Paulo, Sao Paulo, Brazil;

³The JONES Group/JMI Laboratories, North Liberty, IA; ⁴Tufts University School of Medicine, Boston, MA, USA

Received 5 July 2002; returned 26 July 2002; revised 20 August 2002; accepted 20 August 2002

The gene encoding the metallo- β -lactamase SPM-1 was cloned from a genomic library of *Pseudomonas aeruginosa* strain 48-1997A. The insert carrying *spm-1* possessed a GC content of 47%, indicating that it is of non-*Pseudomonas* origin. Upstream of *spm-1* there is a small open reading frame (ORF), which is homologous to the LysR family of proteins (69% identity to the LysR protein from *Salmonella enterica* serovar *Typhimurium*). Downstream of *spm-1* there is the start of an ORF, the product of which shows close homology with the GroEL-type proteins from *Xanthomonas campestris*. No transmissible element could be identified upstream or downstream of *spm-1*. The *spm-1* gene is carried on a plasmid that can transform both *Escherichia coli* and *P. aeruginosa* to ceftazidime resistance. SPM-1 contains the classic metallo- β -lactamase zinc-binding motif HXHXD and shows the highest identity (35.5%) to IMP-1. SPM-1 is a distinctly different metallo- β -lactamase from VIM and IMP and, accordingly, represents a new subfamily of mobile metallo- β -lactamases. The predicted molecular weight of the protein was 27 515 Da, significantly higher than that of IMP (25 041 Da) or VIM (25 322 Da). SPM-1 possesses a unique loop of 23 residues that accounts for the higher molecular mass.

Keywords: metallo- β -lactamase, SENTRY, *Pseudomonas aeruginosa*

Introduction

The advent of carbapenems into clinical practice heralded a new treatment option for irradiating serious bacterial infections caused by cephalosporin- and penicillin-resistant bacteria.¹ However, carbapenem resistance has now been observed in Enterobacteriaceae and in non-fermenter species such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. The common form of resistance is through either lack of drug penetration (i.e. porin mutations and efflux pumps) and/or carbapenem-hydrolysing β -lactamases. Based on molecular studies, two classes of carbapenem-hydrolysing enzymes have been described: serine enzymes possessing a serine moiety at the active site; and metallo- β -lactamases (class B),

requiring divalent cations, usually zinc, as metal cofactors for enzyme activity.^{2,3}

In 1991, a report of a new plasmid-mediated metallo- β -lactamase, IMP-1, in a *P. aeruginosa* isolate caused great concern due to the potential risk of IMP being disseminated widely to other bacterial species.⁴ A 1996–1997 survey of IMP-1-producing Gram-negative bacteria in Japan showed that 144 of 3222 (4.4%) *Serratia marcescens* strains produced IMP-1 through the acquisition of plasmids carrying the *imp* gene.⁵ For many years, the detection of IMP-1-producing isolates was restricted to Japan, but recently the appearance of other IMP-type enzymes has been reported throughout South-East Asia, including Hong Kong and Singapore.^{6,7} An *imp* allelic variant, which encodes IMP-2, was detected in an

*Corresponding author. Tel: +44-117-928-7522; Fax +44-117-928-7896; E-mail: mark.toleman@bristol.ac.uk

Acinetobacter baumannii strain isolated in Italy, the first European example.⁸ A novel family of class B metallo- β -lactamases, the VIM family (VIM-1, VIM-2 and VIM-3 enzymes), was described in *P. aeruginosa* and *Acinetobacter* spp. in Europe.^{9–11} The *vim* gene, like the *imp* gene, is carried on mobile gene cassettes inserted into a class 1 integron. The class 1 integrons are the most common way in which resistant gene cassettes are able to move from one bacterium to another and involve recombination sites, known as 59 bp elements.¹² Although an IMP variant has been reported from Canada, to date there are no reports of mobile metallo- β -lactamases being reported from the USA or Latin America.¹³

In 1999, a 4-year-old female patient was diagnosed with acute lymphoblastic leukaemia and was subsequently admitted to hospital in January 2001 (she also suffered a relapse in November 2000) after her third cycle of chemotherapy at the Instituto de Oncology Pediatrica (Sao Paulo, Brazil). She became neutropenic and presented with episodes of high fever. She was initially treated with ceftriaxone (50 mg/kg/day) and amikacin (15 mg/kg/day) for an ethmoid sinus infection. In early February she had a consolidation of the lower right lobe, and ceftriaxone was replaced by ceftazidime (50 mg/kg/day) for the treatment of suspected pneumonia. Later that month, a urine culture showed a pure growth ($>10^5$ cfu/mL) of a *P. aeruginosa* (designated 48-1997A) susceptible only to polymyxin B, and ceftazidime was replaced by polymyxin B. Five days later, an identical *P. aeruginosa* isolate exhibiting the same antimicrobial susceptibility profile was isolated from a blood culture. The patient died the following day due to septic shock. As part of the SENTRY programme screening protocol of multidrug-resistant *P. aeruginosa* strains worldwide, this isolate was tested with the Etest metallo- β -lactamase strip and, on giving a positive phenotype, was evaluated further.¹⁴ Here, we report the molecular characterization of a mobile metallo- β -lactamase in *P. aeruginosa* from South America as part of the SENTRY antimicrobial surveillance programme.

Materials and methods

Bacterial strains/plasmids

P. aeruginosa 48-1997A was a clinical isolate from Sao Paulo as described above. *Escherichia coli* strain DH5 α [*supE44* Δ *lacU169* (F80*lacZ* Δ M15) *hsdR17* *recA1* *endA1* *gyrA96* *thi-1* *relA1*] was used as the host strain for expressing the cloned β -lactamase gene. Positive controls for the IMP- and VIM-type metallo- β -lactamases were *P. aeruginosa* strains carrying the respective genes. The genomic library was generated in the cloning vector pK18 as described previously.^{15,16}

Determination of MICs

Mid-log phase grown cultures were diluted to 10^{-4} in water. Ten microlitres from each dilution was spotted on to dried nutrient Iso-Sensitest agar, containing serial dilutions of the appropriate antibiotic, using a multipoint inoculator. After 24 h incubation at 37°C, the MIC was determined as the lowest concentration of antibiotic that inhibited the growth in those dilutions that, when inoculated on to nutrient agar containing no antibiotic, gave rise to single colonies.

Metallo- β -lactamase expression in *P. aeruginosa* 48-1997A and from recombinant DNA in *E. coli*

Cellular extracts of *P. aeruginosa* 48-1997A pre-incubated with EDTA (20 mM) or a serine β -lactamase inhibitor (BRL42715 at 5 μ M) had hydrolytic activity determined for cloxacillin, oxacillin, penicillin, ampicillin, imipenem, meropenem, cefaloridine, ceftazidime and nitrocefin. The assays were carried out as described previously by measuring the breakdown of the substrate at a specific wavelength for that β -lactam, except for nitrocefin, where the accumulation of product was measured at 482 nm.¹⁷ Specific activity was measured as nanomoles of substrate hydrolysed/min/mg of protein.

Isoelectric focusing

Isoelectric focusing (IEF) was carried out as described previously.¹⁷ β -Lactamases from *P. aeruginosa* 48-1997A and recombinant clones expressed in *E. coli* were visualized by staining the IEF gels with 100 μ M nitrocefin. Confirmation of the metallo- β -lactamase pI value was performed by pre-incubating the crude cell extract with either 20 mM EDTA or 5 μ M BRL42715 and repeating the IEF.

PCR screening for *vim* and *imp* metallo- β -lactamase genes

For amplification using primers based on the conserved regions of the *imp* and *vim* genes, PCR analysis was carried out using AB-gene Expand Hi-fidelity master mix containing a mix of *Pfu*/non-proofreading *Taq* polymerases and dNTPs. Primers were used at 10 pM concentrations, and 1 μ L of bacterial culture ($OD_{600} = 1$) was used as a template. Cycling parameters were 95°C for 5 min followed by 30 cycles of 95°C for 1 min, annealing at 40°C for 1 min and extension 68°C for 1 min and ending with a 5 min incubation at 68°C. PCR products were visualized by electrophoresis on 0.8% agarose gels in Tris boric acid/EDTA buffer (pH 7.0) and stained with 1% ethidium bromide. The following primers were used for *vim* and *imp* PCR screening (reading 5'–3') and were based on consensus sequences for each of the genes.^{4,6,8–10} The sequences are as follows: *vim* forward,

Molecular characterization of SPM-1

GTCTATTTGACCGCGTC; *vim* reverse, CTAACAAC-GACTGAGCG; *imp* forward, ATGAGCAAGTTATCTG-TATTC; and *imp* reverse, GTCGCAACGACTGTGTAG.

Recombinant DNA methodology

Genomic DNA was isolated from *P. aeruginosa* strain 48-1997A by the cetyl-tri-ammonium bromide method.¹⁶ Plasmids were purified by the alkaline lysis method using the Qiagen miniprep kit. For construction of the genomic library, size fractionated *Sau*3AI fragments (>1 kb) were purified after gel electrophoresis using a Qiagen gel purification kit. Five micrograms of purified genomic fragments were ligated to 1 µg of pK18 that had previously been linearized and dephosphorylated using *Bam*HI and calf intestinal alkaline phosphatase, respectively. The ligation mixture was subsequently dialysed and used to transform *E. coli* DH5α by electroporation using a Bio-Rad Gene Pulser. Plating of the library on to X-gal (30 mg/L) and kanamycin (25 mg/L) plates yielded in excess of 500 000 recombinants per 500 ng of recombinant DNA.

DNA sequencing and sequence analysis

Sequencing was carried out on both strands by the dideoxy-chain termination method with a Perkin Elmer Biosystems 377 DNA sequencer. Sequence analysis was carried out using the Lasergene DNASTAR software package. Sequence alignments were done using Clustal W and PAM 250 matrix. The pI value of the protein was determined using DNASTAR protean. Phylogenetic tree analysis was obtained using DNASTAR. The length of each pair of branches represents the distance between sequence pairs. The scale beneath the tree measures the distance between sequences (DNASTAR, London, UK).

Results

Phenotypic expression of a metallo-β-lactamase from *P. aeruginosa* 48-1997A

P. aeruginosa strain 48-1997A was resistant to all β-lactams except for aztreonam, which had an MIC of 4 mg/L. More often than not, the level of resistance was very high (>256 mg/L). Hydrolytic activities of cellular extracts pre-incubated with and without BRL42715 are shown in Table 1. The addition of EDTA and BRL42715 to cellular extracts inhibited the hydrolysis of meropenem by 93% and 1.4%, respectively. These data indicate that isolate 48-1997A possesses a broad-spectrum metallo-β-lactamase displaying penicillinase, cephalosporinase and carbapenemase activities.

IEF counterstained with nitrocefim was used to determine the pI value of strain 48-1997A enzymes. This index strain possessed two enzymes: a serine enzyme (pI, 6.9) inhibited by

BRL42715; and a second enzyme (pI, 7.5) that is sensitive to EDTA and resistant to BRL42715 inhibition.

Screening for known mobile metallo-β-lactamase genes

To determine whether this strain was an *imp* or *vim* derivative, primers based on conserved regions (aligning the sequences of *vim* 1–3 and *imp* 1–9) of these genes were used in a low stringent PCR screen (annealing at 40°C). Positive controls were *P. aeruginosa* containing *imp* or *vim*. Screening with primers based on *imp* and *vim* conserved regions was negative, whereas the *imp* and *vim* controls were positive, implying that the metallo-β-lactamase gene from *P. aeruginosa* 48-1997A was not a close derivative of *imp* or *vim*.

Cloning of the metallo-β-lactamase gene *spm-1* from *P. aeruginosa* 48-1997A

The gene encoding the metallo-β-lactamase was isolated from a genomic library of *P. aeruginosa* strain 48-1997A constructed in the plasmid vector pK18 and transformed into the *E. coli* host DH5α by screening on nutrient agar plates containing ceftazidime (6 mg/L) and kanamycin (25 mg/L). Twelve colonies were isolated, and subsequent analysis determined ceftazidime MICs in excess of 128 mg/L. In the presence of EDTA (10 mM), the ceftazidime MICs dropped to <4 mg/L. Restriction analysis of the plasmids contained in each colony gave insert sizes ranging from 2 to 7 kb. One clone, 24-S, containing an insert of 2.2 kb, was further analysed by sequencing. The gene encoding the enzyme mediating ceftazidime resistance was designated *spm-1* (São Paulo metallo-β-lactamase).

Table 1. Hydrolytic activities (nmol/min/mg of protein) of *P. aeruginosa* 48-1997A with and without the serine-β-lactamase, BRL42715, and *E. coli* (24-S) expressing SPM-1 (figures in brackets are percentage values taken as imipenem having a relative value of 100)

β-Lactam	Hydrolytic activity of crude cell extracts		
	48-1997A	48-1997A with BRL42715	<i>E. coli</i> (24-S)
Meropenem	468 (600)	460 (708)	1306 (607)
Imipenem	78 (100)	65 (100)	215 (100)
Penicillin	444 (569)	336 (517)	1115 (519)
Ampicillin	660 (846)	221 (340)	705 (328)
Cefaloridine	710 (910)	288 (443)	910 (423)
Ceftazidime	55 (71)	20 (37)	58 (27)
Cloxacillin	18 (24)	9.5 (15)	38 (14)
Oxacillin	21 (26)	8.4 (13)	34 (14)
Nitrocefim	98 (116)	2.4 (3.7)	6.1 (2.9)

Biochemical analysis of recombinant SPM-1

Cellular extracts from *E. coli* 24-S (expressing SPM-1) are shown in Table 1. These values, when compared with the crude cell extracts from strain 48-1997A [in the presence of BRL42715 (5 μ M)], show almost identical ratios of hydrolysis for the different β -lactams, indicating that the enzyme expressed from the cloned gene is responsible for the activity seen in strain 48-1997A when pre-incubated with BRL42715 (inhibiting serine β -lactamase activity). The pI value of SPM-1 expressed in *E. coli* was 7.5 (data not shown), confirming the IEF analysis from the crude cell extracts on *P. aeruginosa* 48-1997A.

*Sequence analysis of *spm-1* and its genetic context*

The DNA insert carried by 24-S possessed a GC content of 47%, indicating that it is of non-*Pseudomonas* origin, which in comparison is ~66% (http://www.sanger.ac.uk/Projects/P_fluorescens/). 24-S contained an open reading frame (ORF) encoding a putative protein of 264 amino acids displaying homology with previously cloned metallo- β -lactamases: namely, IMP-type proteins. The sequence of *spm-1* has been deposited in the EMBL database with the accession number AJ492820. The N-terminus of the predicted protein shows typical features of bacterial signal peptides: namely, a hydrophobic portion of ~13 amino acids, preceded by five highly charged molecules that target protein secretion to the periplasm. Sequencing of the N-terminus of the mature protein identified the cleavage site of the signal peptide between serines 18 and 19. The predicted molecular weight of the protein was 27 515 Da, which is significantly higher than that of IMP (25 041 Da) or VIM (25 322 Da).^{8,18} The theoretical pI value of SPM-1 (not including the leader sequence) is calculated to be 8.91, which is significantly different from the actual value of 7.5 measured by IEF. This phenomenon has been reported for other β -lactamases.¹⁷

Upstream of *spm-1* there is a small ORF, designated ORF2, which is homologous to the LysR family of proteins (69% identity to the LysR protein from *Salmonella enterica* serovar *Typhimurium*) (Figure 1). The spacing between the ORFs is 150 bp. ORF2 is predicted to be transcribed in the opposite direction from that of *spm-1*; however, the protein appears

to be truncated and therefore non-functional. Downstream of *spm-1* there is the start of an ORF, designated ORF3. ORF3 is predicted to be transcribed in the same direction as *spm-1*, the product of which shows close homology with the GroEL-type proteins from *Xanthomonas campestris* and *S. enterica* with 73% and 65% identity, respectively.¹⁹ Within the cloned insert, no sequence showing any degree of homology with integrons was found. The insert carried by 24-S was confirmed by PCR to be that of strain 48-1997A.

Alignment and homology with other class B enzymes

The protein sequence of SPM-1 contains the metallo- β -lactamase zinc-binding motif HFHLD, as well as other residues that have been implicated in binding two zinc ions (Figure 2). The mature protein of SPM-1 showed the highest identities to the following metallo- β -lactamases: 35.5% to IMP-1, 32.2% to ImiS, 32.1% to CphA, 30% to BCII and 27% to CcrA.²⁰⁻²⁴ When aligned with the other class B β -lactamases, SPM-1, similarly to the *Aeromonas* metallo- β -lactamases and L1 from *Stenotrophomonas maltophilia*, has a loop that is not present in the other class B enzymes (Figure 2).^{21,26} In SPM-1 this loop comprises 23 amino acids, whereas in the *Aeromonas* spp. class B enzymes and L1 it is 17 and 10 amino acids, respectively. Although the highest identity is seen with IMP-1, SPM-1 shares more identity with the C-terminus of IMP-1 than with the N-terminus. From the N-terminus to the start of (but not including) the loop, SPM-1 exhibits 31% identity to IMP-1, whereas, from (but not including) the loop to the C-terminus, SPM-1 exhibits 47% identity to IMP-1.⁴ As judged by the sequence homology alone, SPM-1 should be classified with the group 3a β -lactamases.

The phylogenetic tree (Figure 3) shows SPM-1 aligning with the IMP-type class B enzymes rather than with the others. However, the clustal weighting clearly shows that SPM-1 is different and only distantly related to the IMP-type enzymes. Although the next highest identity was seen with the *Aeromonas* spp. class B enzymes ImiS and CphA,^{21,22} the phylogenetics indicate that SPM-1 is distantly related to these enzymes.

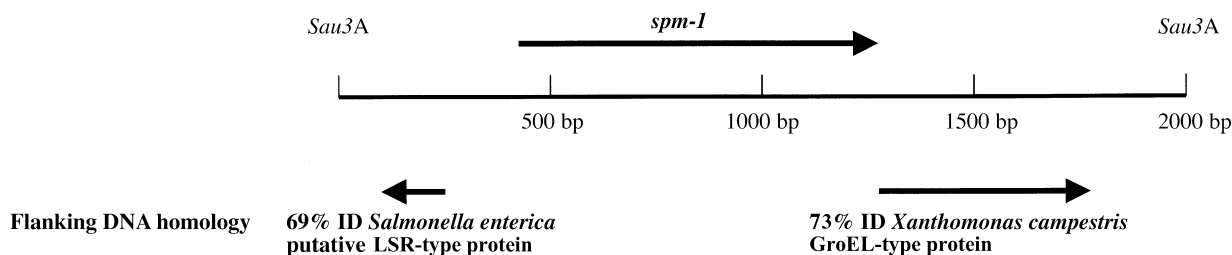


Figure 1. Arrangement of the 2203 bp *Sau3A* chromosomal insert of *P. aeruginosa* 48-1997A. Upstream there is a putative LysR-type regulator that appears to be truncated, and downstream there is a GroEL-type protein similar to that of *X. campestris*.¹⁹

Molecular characterization of SPM-1

SPM-1	-----SDHVDLPYNLTKIDSDVVFVVDTRDFYSS-----NVLVAKMLDGT
IMP-1	-----AESLFDLKIETKLDGQVYVHTSFEVNGWGVVPKHGLVVLVNAE
VIM-1	SPLAHSGBSPGEYPTVNEIPVGEVRLYQIADGVNSHIATQSFDDG-AVYPSNGLIVRDGDE
BCII	-----SQVKEKTVIKNETGTIISIQLNKNVWVHTELGFSFNG-EAVPSNGLVNLNTSKG
CcrA	-----AQKSVKISDDISITQLSDKVVYVSLAEITBEGWGMVPSNGMIVINNHQ
ImiS	-----AGMSLTQVSGPVYVVEDNYVQE-----NSMVFYFGAKG
BlaB	-----QENPDVKIDKLDNLYVYTYNTYFNG-TKYAANAVYLVTDKG
L1	-----AEVPLPQLRAYTVDAWSLQPMAPLQIADHTWQIGTEDLTALLVQTPDGA
SPM-1	VVIVSSPFENLGTQTLMDVAKTKMKPKKVVAINTHFHLDTGGNEIYKMKGAETWSSDLT
IMP-1	AYLIDTPTAKDTKELVTVFVFER-GYKIKGSISSPHSDSTGGIEWLNSRSIPTYASELT
VIM-1	LLIIDTAWGAKNTAALLAEIEKQIGLFPVTRAVSTPHDDRGGVVDVLRAGVATYASPS
BCII	LVLVDSWDDKLTKELEIEMVEKFKQKRVTDV I I THAHADRI GGI KTLKERGI KAHSTALT
CcrA	AALLDTPINDAQTEMLVNVVTDLSLHAKVTFIPNHWHGDCI GGLGYLQKRGVQSYANQMT
ImiS	VTVVGTATWPTARELHKLIRKVRKPVLEVINTNYHTDRAGGNAYWKSIGAKVVSTROT
BlaB	VVVIDCPWGEDKFKSFTDEIYKMGKVIIMNIIATHSHDDRRAGGLVYFGKIGAKVYSTKMT
L1	VLLDGGMPQASHLLDNMKARGVTPTRDLRLILLSHAHADHAGPVAELKRRTGAKVAANAE
* * *	
SPM-1	KQLRLEENKDKRIKAAEFYKNEDELRRLSSHPVPADNVFDLQKGVFSFNSNELVEVSFP
IMP-1	NELL---KDDGKQVATNSFS-----GVNYWLVKNKIEVFYF
VIM-1	RRLAEEAGNEIPTHSLLEGL-----SSSGDAVRFGPVLFYF
BCII	AELA---KKNGYEELPLGLDQ-----TVNLFKFGNMKVETFFYP
CcrA	IDLA---KEKGLVPEHGFT-----DSLTVSLDGMPLQCYLA
ImiS	RDLM---KSDWAEIVAFTRKGLPE-----YPDLPLVLPNVVHDDGFTLQEGKVRAFYA
BlaB	DSILA---KENKPRAYTFD-----NNKSPFKVKGSEFQVYYP
L1	SAVLLARGSSDDLHFGDGI T Y P P-----ANADRIVMDGEVITVGGIVPTAHT
SPM-1	GPAHSPDNVVVYFPKCKLLFGGCMIKPKE---LGYLGDANVKAWPDSARRLKK--FDAKI
IMP-1	GPGHTPDNVVWLPERKILFGGCFIKPYG---LGNLGDANI EAWPKSAKLLKSKYGKAKL
VIM-1	GAAHSTDNLVVYVPSANVLYGCCAVHLSSTSAAGNVADADLAEWPTSVERIQKHYPEAEV
BCII	GKGHTEDNIVVWLPQYNIIVGGCLVKSTSAKDLGNVADAYVNEWSTSIENVLKRYRINIA
CcrA	GGGHATDNIVVWLPTEINLFGGCMKLDNQAISIGNISDADVTAWPKTLDKVKAKFPARSY
ImiS	GPAHTPDGI FVYFPDEQVLYGNCILKEKL---GNLSFADVKAYPQTLERLKAAMKLPITK
BlaB	GKGTADNVVWVFPKCKVLVGGCIIKSDASDLDGYIGEAYVNDWTSVHNIQQKFSGAQY
L1	MAGHTPGSTAWTWDTRNGKPVRIAYADSLSAPGYLQGNPRYPHILIEDYRRSFATVRAL
*	
SPM-1	----VIPGHGEWGPPEMVKTIKVAEKAAGEMRL-----
IMP-1	----VVPSEHSEVDASLLKLTLEQAVKGLNESKPKSKPSN-----
VIM-1	----VIPGHGLPGGLDLLQHTANVVAHAKNRSVAE-----
BCII	----VVPGHGEVGDGKLLHHTLDDLK-----
CcrA	----VVPGHGIDYGGTELEIHTKQIVNQYIESTSKP-----
ImiS	----VIGGHDSPLHGPELIDHYEALIKAAPQS-----
BlaB	----VVAGHDDWKDQRSIQRTLDLINEYQQKQKASN-----
L1	PCDVLITPHFGASNWDYAAGARAGAKALTKCAYADAAEQKFDGQLAKETAGAR
*	

Figure 2. Amino acid sequence alignment of SPM-1 with IMP-1, VIM-1, BCII, CcrA, ImiS, BlaB and L1.^{8,20,21,23–26} Conserved residues coordinating the zinc ions are denoted with an asterisk. The 23-amino-acid loop of SPM-1 is denoted in bold.

Discussion

E. coli carrying recombinant clones of *spm-1* could be selected for on media containing ceftazidime at high concentrations. However, like *vim* and *imp*, *spm-1* when expressed in *E. coli* does not confer resistance to the carbapenems

(imipenem and meropenem MICs of 1 and 0.25 mg/L, respectively) or aztreonam (1 mg/L) but does confer resistance to the penicillins and all cephalosporins.

Given the low GC content (47%) of the insert carrying *spm-1*, there is little doubt that the gene has been imported into *Pseudomonas*, despite the fact that attempts to mate it across into either an *E. coli* or *Pseudomonas* recipient failed (data not shown). However, we have determined that *spm-1* is present on a large plasmid and that this can be used to transform both *E. coli* and *P. aeruginosa* to ceftazidime resistance. Both *vim*- and *imp*-type genes have been shown to be associated with class 1 type integrons. However, immediately upstream or downstream of *spm-1* there were no sequences homologous with transmissible elements. One hundred and fifty base pairs upstream of *spm-1* there is a gene encoding a LysR-type protein. The gene context of *spm-1* is similar to the *qacI/LysR* locus associated with the β -lactamase PSE-1 on a multidrug-resistance island found in *S. enterica* serovar *Typhimurium* DT104 (Figure 1).¹⁹

Sequence analysis of SPM-1 shows it to be most similar to IMP-1 but that it contains a loop of 23 residues that explains the higher molecular mass. Interestingly, SPM-1 shows more homology for IMP-1 towards the C-terminus of the protein than to the N-terminus, and this demarcation seems to occur immediately before and after the loop. Although it is mere speculation, it is interesting to raise the possibility that SPM-1 could be a hybrid protein of two ancestral class B type enzymes, one being more IMP-1-like than the other. The alignment of SPM-1 with the other class B enzymes clearly shows that SPM-1 contains the classic HXHXD motif as well as key histidine (positions 165 and 221) and cysteine (position 184) residues that are capable of coordinating two zinc molecules per molecule of enzyme.

The data indicate that *spm-1* is a novel metallo- β -lactamase gene that is significantly different from the IMP or VIM groups of enzymes. Although we could not identify any transmissible element adjacent to *spm-1*, the gene is likely to be mobile. Crude enzyme kinetics indicate that SPM-1 can hydrolyse all classes of β -lactam antibiotics. Rather like IMP

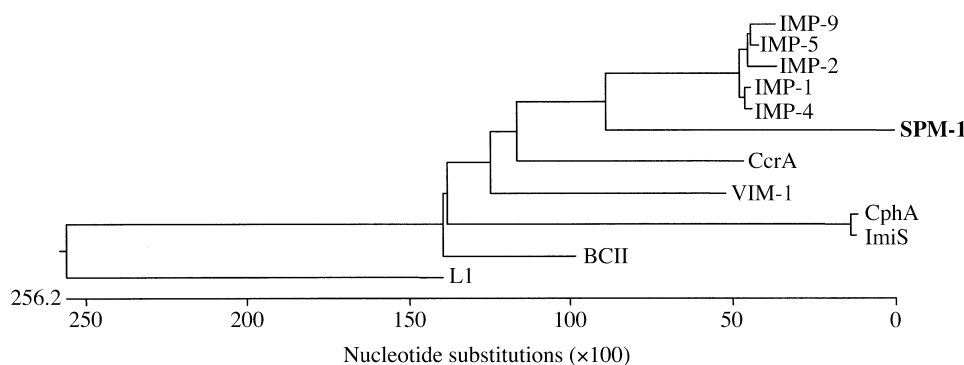


Figure 3. Phylogenetic tree showing clustal weighting and relatedness of SPM-1 to other metallo- β -lactamases. SPM-1 is clustally aligned with the IMP-type β -lactamases rather than with the class B enzymes.

and VIM, SPM-1 poses a significant threat to metallo- β -lactam regimens that may be used to treat systemic infections, notwithstanding the fact that there is no clinically available metallo- β -lactamase inhibitor.

Acknowledgements

We would like to thank J. Kirby, K. Gordon and P. Rhomberg for technical support in the screening process. We would also like to thank the Medical Research Council for supporting T. A. M. and the Biotechnology and Biological Sciences Research Council for supporting A. M. S. This work was sponsored partly by the British Society for Antimicrobial Chemotherapy. The SENTRY programme was funded by an educational/research grant from Bristol-Myers Squibb.

References

- Kroop, H., Sundelf, J. G., Kahan, J. S., Kahan, F. M. & Birnbaum, J. (1985). MK0787 (*N*-formimidoyl thienamycin): evolution of in-vitro and in-vivo activities. *Antimicrobial Agents and Chemotherapy* **17**, 993–1000.
- Livermore, D. M. & Woodford, N. (2000). Carbapenemases: a problem in waiting? *Current Opinions in Microbiology* **3**, 489–95.
- Page, M. I. (2002). Understanding metallo- β -lactamases. *ASM News* **68**, 217–21.
- Arakawa, Y., Murakami, M., Suzuki, K., Ito, H., Wacharotayan-kun, R., Ohsuka, S. *et al.* (1995). A novel integron-like element carrying the metallo- β -lactamase gene blaIMP. *Antimicrobial Agents and Chemotherapy* **39**, 1612–5.
- Senda, K., Arakawa, Y., Ichiyama, S., Nakashima, K., Ito, H., Ohsuka, S. *et al.* (1996). PCR detection of metallo- β -lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum β -lactams. *Journal of Clinical Microbiology* **34**, 2909–13.
- Chu, Y. W., Afzal-Shah, M., Houang, E. T., Palepou, M. I., Lyon, D. J., Woodford, N. *et al.* (2001). IMP-4, a novel metallo- β -lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. *Antimicrobial Agents and Chemotherapy* **45**, 710–4.
- Koh, T. H., Babini, G. S., Woodford, N., Sng, L. H., Hall, L. M. & Livermore, D. M. (1999). Carbapenem-hydrolysing IMP-1 β -lactamase in *Klebsiella pneumoniae* from Singapore. *Lancet* **353**, 2162.
- Lauretto, L., Riccio, M. L., Mazzariol, A., Cornaglia, G., Amicosante, G., Fontana, R. *et al.* (1999). Cloning and characterization of bla_{VIM}, a new integron-borne metallo- β -lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. *Antimicrobial Agents and Chemotherapy* **43**, 1584–90.
- Tsakris, A., Pournaras, S., Woodford, N., Palepou, M. F., Babini, G. S., Douboyas, J. *et al.* (2000). Outbreak of infections caused by *Pseudomonas aeruginosa* producing VIM-carbapenemase in Greece. *Journal of Clinical Microbiology* **38**, 1290–2.
- Poirel, L., Naas, T., Nicolas, D., Collet, L., Bellais, S., Cavallo, J. D. *et al.* (2000). Characterization of VIM-2, a carbapenem-hydrolysing metallo- β -lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrobial Agents and Chemotherapy* **44**, 891–7.
- Franceschini, N., Caravelli, B., Docquier, J. D., Galleni, M., Frere, J. M., Amicosante, G. *et al.* (2000). Purification and biochemical characterization of the VIM-1 metallo- β -lactamase. *Antimicrobial Agents and Chemotherapy* **44**, 3003–7.
- Hall, R. M., Brooks, D. E. & Stokes, H. W. (1991). Site specific insertion of genes into integrons: role of the 59bp element and determination of the recombination crossover point. *Molecular Microbiology* **5**, 1941–59.
- Gibb, A. P., Tribuddharat, C., Moore, R. A., Louie, T. J., Krulicki, W., Livermore, D. M. *et al.* (2002). Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla(IMP) allele, bla(IMP-7). *Antimicrobial Agents and Chemotherapy* **46**, 255–8.
- Walsh, T. R., Bolmström, A., Qwärnström, A. & Gales, A. (2002). Evaluation of a new Etest to detect metallo- β -lactamases in routine clinical testing. *Journal of Clinical Microbiology* **40**, 2755–9.
- Pridmore, R. (1987). New and versatile cloning vectors with kanamycin resistance markers. *Gene* **56**, 309–12.
- Walsh, T. R., MacGowan, A. P. & Bennett, P. M. (1997). Sequence analysis and kinetics of the cloned L2 serine β -lactamase from *Stenotrophomonas maltophilia*. *Antimicrobial Agents and Chemotherapy* **41**, 273–81.
- Walsh, T. R., Emery, D. C., MacGowan, A. P. & Bennett, P. M. (1996). Enzyme kinetics and biochemical analysis of ImiS, a metallo- β -lactamase from *Aeromonas sobria* 163a. *Journal of Antimicrobial Chemotherapy* **37**, 423–31.
- Laraki, N., Franceschini, N., Rossolini, G. M., Santucci, P., Meunier, C., de Pauw, E. *et al.* (1999). Biochemical characterization of the *Pseudomonas aeruginosa* 101/1477 metallo- β -lactamase IMP-1 produced by *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **43**, 902–6.
- Boyd, D., Peters, G. A., Cloeckert, A., Boumedine, K. S., Chaslus-Dancla, E., Imberechts, H. *et al.* (2001). Complete nucleotide sequence of a 42-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar *Typhimurium* DT104 and its identification in phage type DT120 and serovar *Agona*. *Journal of Bacteriology* **183**, 5725–32.
- Osano, E., Arakawa, Y., Wacharotayan-kun, R., Ohta, M., Horii, T., Ito, H. *et al.* (1994). Molecular characterization of an enterobacterial metallo- β -lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. *Antimicrobial Agents and Chemotherapy* **38**, 71–8.
- Walsh, T. R., Neville, W. A., Tolson, D., Payne, D. J., Bateson, J., MacGowan, A. P. *et al.* (1998). Nucleotide and amino acid sequences of the metallo- β -lactamase, ImiS, from *Aeromonas veronii* bv. *sobria*. *Antimicrobial Agents and Chemotherapy* **42**, 436–9.
- Massidda, O., Rossolini, G. M. and Satta, G. (1991). The *Aeromonas hydrophila* *cphA* gene: molecular heterogeneity among Class B metallo- β -lactamases. *Journal of Bacteriology* **173**, 4611–7.
- Hussain, M., Carlino, A., Madonna, M. J. & Lampen, J. O. (1985). Cloning and sequencing of the metalloprotein β -lactamase II gene of *Bacillus cereus* 569/H in *Escherichia coli*. *Journal of Bacteriology* **164**, 223–9.

Molecular characterization of SPM-1

24. Thompson, J. S. & Malamy, M. H. (1990). Sequencing the gene for an imipenem-cefoxitin-hydrolyzing enzyme (CfiA) from *Bacteroides fragilis* TAL2480 reveals strong similarity between CfiA and *Bacillus cereus* β -lactamase II. *Journal of Bacteriology* **172**, 2584–93.
25. Rossolini, G. M., Franceschini, N., Riccio, M. L., Mercuri, P. S., Perilli, M., Galleni, M. *et al.* (1998). Characterization and sequence of the *Chryseobacterium (Flavobacterium) meningosepticum* carbapenemase: a new molecular class B β -lactamase showing a broad substrate profile. *Biochemical Journal* **332**, 145–52.
26. Walsh, T. R., Hall, L., Assinder, S. J., Nichols, W. W., Cartwright, S. J., MacGowan, A. P. *et al.* (1994). Sequence analysis of the L1 metallo- β -lactamase from *Xanthomonas maltophilia*. *Biochimica et Biophysica Acta* **1218**, 199–201.

